Technical methods

The effect of autoclaving characteristics on the recovery of serum vitamin B₁₂ determined by a radioisotope dilution method

D. H. ORRELL AND A. D. CASWELL *From the Group Pathology Laboratory, Lakin Road, Warwick*

Radioisotope methods of serum B₁₂ determination require that the binding activity of serum proteins is completely destroyed so that all the vitamin is freed from them. If this is not done then only a proportion of the total vitamin B₁₂ is available to dilute the labelled vitamin added during the test, and, furthermore some of this label may itself become bound to the remaining binding proteins. Using the method of Raven, Robson, Walker, and Barkhan (1969) it has been found that excessive autoclaving of serum samples produces low results. It is therefore important to choose autoclaving characteristics which give the best recovery of the vitamin. This cannot be determined by the standard method of adding known increments to serum and measuring the rise in concentration because the added vitamin B₁₂ is not bound to the transcobalamins in the same manner as endogenous vitamin. An alternative approach is to define the conditions of autoclaving which give the most reproducible results and this has been used in the investigation.

Method

A pool of serum was prepared from a large number of hospital patients not suffering from carcinoma, leukaemia, or liver disease and who had not received any vitamin B₁₂ therapy. Aliquots, each of 20 ml, of this pool were stored at -20°C. The technical methods used followed those described by Raven *et al* (1969) except that the intrinsic factor was obtained from Armour Pharmaceuticals, the activated charcoal was Norit OL from Hopkins and Williams, and a vertical laboratory autoclave (British Sterilizer Co) giving alternative pressures of 5 lb and 20 lb was preferred to a domestic pressure cooker.

Received for publication 20 April 1971.

Results and Comment

The mean vitamin B₁₂ concentration found within each batch is shown in Figure 1. The concentration falls with increasing periods of autoclaving and is more rapid at the higher pressure. It also appears that the standard deviation is greater when the batch is autoclaved at the higher pressure for more than five minutes.

These results suggest that the optimum conditions are to autoclave at 5 lb pressure for 15 minutes.

A simple explanation for the fall in B₁₂ concentration described would be that excessive heating

Fig. 1 The mean vitamin B₁₂ concentration of a pool serum when autoclaved at 5 lb or 20 lb pressure for 5, 10, 15, 20, or 30 minutes. Two standard deviations about each mean are indicated by interrupted lines.

Fig. 2 The mean net counts/200 sec in the supernatants of 18 replicate test sets of test tubes prepared from pool serum when autoclaved at 5 lb or 20 lb for 5, 10, 15, 20, or 30 minutes. Two standard deviations about each mean are indicated by dotted lines.

destroys some of the vitamin. In fact the radioactivity in the supernatant falls slightly (Fig. 2). The fall in calculated concentration of vitamin B₁₂, however, is due mainly to the large fall in the radioactivity in the supernatants prepared from sera artificially freed from B₁₂ (intrinsic factor control, Fig. 3). Two possible explanations for this pheno-

Fig. 3 The net counts/200 sec in the supernatants of two sets of intrinsic factor control tubes when autoclaved at 5 lb or 20 lb for 5, 10, 15, 20, or 30 minutes.

menon are that binding of vitamin B₁₂ by intrinsic factor is impaired by degradation products in autoclaved serum or the albumin-coated charcoal adsorbs more of the intrinsic factor bound vitamin B₁₂ in the presence of excessively autoclaved serum.

Reference

- Raven, J. L., Robson, M. B., Walker, P. L., and Barkhan, P. (1969) Improved method for measuring vitamin B₁₂ in serum using intrinsic factor, ⁵⁷Co B₁₂, and coated charcoal. *J. clin. Path.* 22, 205-211.